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Assessing the bioefficacy of conventional solvent and supercritical fluid extracts of green tea to alleviate lifestyle related dysfunctions

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Abstract

Background: Nowadays, consumers are focusing on therapeutic diets that not only serve the purpose of basic nutrition but also possess varied ingredients to fight against free radical mediated malfunctions especially cardiac disorders and type 2 diabetes. In this context, green tea polycatechins need to be introduced in the dietary regimens as per their effective level. Keeping in view, the present study was aimed to assess the hypocholesterolemic and hypoglycemic potential of conventional and supercritical fluid extracted green tea polycatechins, using male Sprague Dawley rats.

Methods: The in vivo study was carried out for 60 days to check the response of green tea extract based diets on animal body and serum biomarkers. Three animal trials were conducted; Study I (Normal rats), Study II (hyperglycemic rats) and Study III (hypercholesterolemic rats). Each study was further sectioned into three groups based on dietary modules; control diet, functional diet (enriched with solvent extracted green tea polycatechins) and nutraceutical diet (carrying supercritical fluid extracted green tea polycatechins).

Results: The decrease in cholesterol, triacylglycerol, low density lipoprotein-cholesterol (LDL-c) and glucose was viewed among all groups along with increment in high density lipoproteins-cholesterol (HDL-c) and insulin levels. Furthermore, green tea nutraceutical diet showed an upper hand in ameliorating lifestyle related disorders as compared to functional diet. Though, marked decrement in lipid biomarkers especially cholesterol (15.22%) and LDL-c (20.50%) were found in Study III (hypercholesterolemic rats), whereas highest suppression in glucose (12.71%) and improvement in insulin (8.28%) was obviously recorded in Study II (hyperglycemic rats).

Conclusion: Green tea polycatechins based diets have proven their efficacy in mitigating hypercholesterolemia and hyperglycemia induced by cholesterol and sucrose based dietary regimen.

Keywords: Functional diet, Nutraceutical diet, Green tea, Solvent extract, Supercritical fluid extract, Hyperglycemia, Hypercholesterolemia, Sprague Dawley rats

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Background

Nowadays, consumers are shifting their preferences towards such dietary therapies that could tackle the menace of hyperglycemia, hypercholesterolemia and various oncogenic incidences [1]. In this context, diets rich in phytochemicals such as fruits, vegetables, spices and herbs and tea leaves are getting fame [2, 3]. Green tea (*Camellia sinensis L.)* belongs to *Theaceae* family, originated from China and converging popularity around the globe since seventeenth century. It carries volatile and non-volatile constituents of Ayurvedic potential [4]. Dried green tea leaves enumerate 30% of polyphenolic molecules. Of the total, 70% of the phytonutrients include epigallocatechin gallate, epicatechin gallate, epigallocatechin, epicatechin and gallocatechin [5, 6].

The term diabetes is characterized by two different metabolic states; IDDM (insulin dependent diabetes mellitus) is directly associated with deteriorated insulin activity due to the damaged islets of Langerhans, whereas NIDDM (non-insulin dependent diabetes mellitus) is linked with abnormal signaling pathway of insulin that increases insulin resistance and reduces glucose conversion to glycogen in liver. In this regard, epigallocatechin gallate mainly avert the pathogenesis of insulin resistance thereby free glucose no longer stays in the blood rather converted to glycogen in the body [7, 8]. Various researchers have also demonstrated increase in the activity of antioxidant enzymes like superoxide dismutase, catalase and glutathione oxidase via green tea extract, ultimately over-coming free-radical induced diseases and related complications [9, 10].

Hypercholesterolemia is a condition i.e. associated with elevated LDL-c level i.e. (bad cholesterol), being low in density, plaque formation in arterial walls, intensifying incidences of angina and heart attack. Conversely, improvement in HDL-c to LDL-c ratio is considered safe [11]. In this context, green tea extract has the ability to inhibit HMG-CoA (3 hydroxy-3-methylglutaryl coenzyme-A) reductase by activating AMP-kinase resultantly modulates cholesterol synthesis in the body cells [12]. Furthermore, epigallocatechin gallate is related with reduced cholesterol absorption in the intestinal lumen [13].

A clinical trial (32 hyperlipidemias) showed that three cups of green tea (200 mL) per day has the ability to reduce LDL (15.6%), cholesterol (8.36%) and triacylglycerol (18.14%). The reason might be the presence of polycatechins that possess similar response as that of thromboxane (vasodilator). Additionally, galloyl moiety of polycatechins has the potential to regulate hyper-triacylglycerol level to normative values. Besides, green tea has shown its ability to improve HDL-c (9.66%) and apo-A1 while control LDL and apo-B by excreting free lipid bodies without absorption [12]. Further, Brown et al. [8] found response of epigallocatechin gallate (EGCG) in improving insulin up to 2.97% by deactivating I-kappa kinase i.e. involved in insulin resistance.

Methods

Chemicals and procurement of raw material

The diagnostic kits were used from Sigma-Aldrich, Bioassay (Bioassays Chemical Co. Germany) and Cayman Chemicals (Cayman Europe, Estonia). The dried green tea leaves were purchased from the local market. The leaves were ground and enclosed in zip lock bags at room temperature for experimentation.

Preparation of green tea extracts

The conventional solvent extraction (CSE) was prepared by combining dried green tea with binary solvent system at ratio 1:4: HPLC graded water and organic solvent, aqueous acetone (50 *v*/v). The extract was macerated overnight followed by extraction using orbital shaker (Edmund Buhler Gmg H-Ks 15, Germany) with speed (280 rpm) at 50 min with constant temperature of 50 °C as expressed by Dong et al. [14]. Afterwards, solvent extract was filtered then concentrated using Rotary Evaporator (Eyela, Japan) at 40 ± 5 °C. The catechins were measured as 1325.81 ± 64.85 mg/100 g in dry green tea extract. The supercritical fluid extraction (SFE) of green tea was acquired through SFT-150 system using 99.8% pure CO_2 at 50 °C. After the placement of sample in 100 mL extraction vessel, CO_2 was liquefied at 3000 psi pressure to accelerate the solvation and mass transfer of the desired active moieties. After a certain duration, extract was obtained in the collecting vial manually after reducing the set pressure. The catechins in green tea supercritical fluid extract were found as 7723 mg/100 g in dry green tea extract [15].

Animal studies

Sixty male Sprague Dawley rats were acquired from National Institute of Health (NIH), Islamabad, Pakistan and kept in Animal Room of National Institute of Food Science and Technology, University of Agriculture Faisalabad (NIFSAT-UAF), Pakistan. The investigation was carried out in three studies separately (Table 1). Study I comprised of rats fed on normal diet, whereas in study II and III, high glucose and high cholesterol diets were given, respectively. Each group was having 5 rats, though some animals were sacrificed initially to obtain the baseline values. During the entire bioassessment trials, Animal Room was maintained at a temperature and relative humidity of 23 ± 2 °C and 55 ± 5%, respectively. In each study, functional and nutraceutical extract based diets were provided to their respective groups alongside normal diet was fed to normal animals to assess the efficacy of each treatment on the selected parameters; serum lipid profile, glucose and insulin levels. Besides

Table 1 Efficacy plan

Research plan	Groups	Dietary pattern
Study I	Control	Normal diet
	Functional diet	Conventional solvent extract (CSE) + Normal diet
	Nutraceutical diet	Supercritical fluid extract (SFE) + Normal diet
Study II	Control	High sucrose (40%) diet
	Functional diet	Conventional solvent extract (CSE) + High sucrose (40%) diet
	Nutraceutical diet	Supercritical fluid extract (SFE) + High sucrose (40%) diet
Study III	Control	High cholesterol (1.5%) diet
	Functional diet	Conventional solvent extract (CSE) + High cholesterol (1.5%) diet
	Nutraceutical diet	Supercritical fluid extract (SFE) + High cholesterol (1.5%) diet

Study I (Normal rats); Study II (Hyperglycemic rats); Study III (Hypercholesterolemic rats)

baseline values, the blood was also drawn at 30th day to check the response followed by sacrificing of animals at 60th day. The collected blood in yellow-capped vials was used to probe hyperlipidemic and hyperglycemic perspectives, whereas purple capped vials were employed to assess hematology.

Feed and water intakes

Average feed intake of each group was measured on daily basis by eliminating spilt diet from the total diet given during the whole study period [16]. The water intake for each group was also recorded on daily basis.

Body weight

Increase in body weight of rats from all experimental groups was measured weekly throughout the study period to analyze the effect of functional and nutraceutical diets on body weight.

Serum lipid profile

Serum cholesterol levels of rats were measured using CHOD-PAP method following the protocol of Elwakkad et al. [17]. Whilst, Low Density Lipoproteins-cholesterol (LDL-c) and High Density Lipoproteins-cholesterol (HDL-c) were determined as described by Alshatwi et al. [18]. Furthermore, triacylglycerol levels in the samples were estimated by liquid triglycerides (GPO-PAP) method as illustrated by Mehra et al. [19].

Serum glucose and insulin levels

For each study, the collected sera were evaluated for glucose concentration by GOD-PAP instructions as described by Kim et al. [20], whereas insulin levels of rats were assessed using its respective kits following the method of Ahn et al. [21].

Hematological aspects

Red blood cell (RBC) and white blood cell (WBC) counts were determined by the method of Al Haj et al. [22]. While, platelets estimation was carried out as described by Kamatani et al. [23]. The hematological measurements were carried out using Medonic M Series; Boule Diagnostics Int AB Stockholm, Sweden.

Statistical design

The obtained data for each parameter was subjected to statistical modeling through completely randomized design (CRD) using Statistix 8.1. Furthermore, level of significance was also estimated ($p < 0.05$ and $p < 0.01$) by using analysis of variance (ANOVA) technique followed by LSD multiple comparison tests for means separation [24].

Results

Feed and water intakes

The statistical inference for feed intake depicted significant differences due to diets and time frame throughout the trial in all the three studies. The Fig. 1 showed an increasing trend in feed intake however, more increase was observed in normal (control) group in each study. The functional and nutraceutical diets were less consumed as compared to control groups in all the three studies. In study I (Normal rats), the control, functional and nutraceutical diets varied significantly from week 1st to 8th as 13.46 ± 0.62 to 19.23 ± 0.94, 13.02 ± 0.63 to 18.35 ± 0.89 and 12.95 ± 0.62 to 17.87 ± 0.85 g/rat/day, respectively. Likewise, in study II (Hyperglycemic rats) and study III (Hypercholesterolemic rats), the feed intake varied momentously in control, functional and nutraceutical diets supplemented groups from 14.47 ± 0.71, 14.12 ± 0.65 and 14.01 ± 0.61 to 21.39 ± 1.02, 20.61 ± 1.03 and 20.29 ± 1.02 and 14.01 ± 0.68, 13.84 ± 0.66 and 13.38 ± 0.62 to 20.31 ± 1.01, 19.79 ± 0.96 and 18.99 ± 0.91 g/rat/day, respectively.

The mean squares regarding water intake showed non-momentous variations with respect to diets whilst, differed significantly regarding study duration. The (Fig. 2) demonstrated higher water intake in control groups of all the three studies; study I (Normal rats), study II (Hyperglycemic rats) and study III (Hypercholesterolemic rats) in contrast to the nutraceutical based diets as observed in feed intake pattern because water to feed ratio remained constant under similar conditions. During 60 days, the mean values for control, functional and nutraceutical diets varied from 19.17 ± 0.92, 18.58 ± 0.89 and 18.1 ± 0.87 to 24.73 ± 1.21, 23.59 ± 1.09 and 22.63 ± 1.07 mL/rat/day in study I, from 20.11 ± 0.97, 19.76 ± 0.93 and 19.25 ± 0.91 to 26.34 ± 1.28, 25.49 ± 1.23 and 24.64 ± 1.21 mL/rat/day in case of study II and from 19.85 ± 0.95, 19.24 ± 0.92 and 18.98 ± 0.93 to 25.81 ± 1.24, 24.63 ± 1.21 and 23.92 ± 1.13 mL/rat/day in study III, respectively.

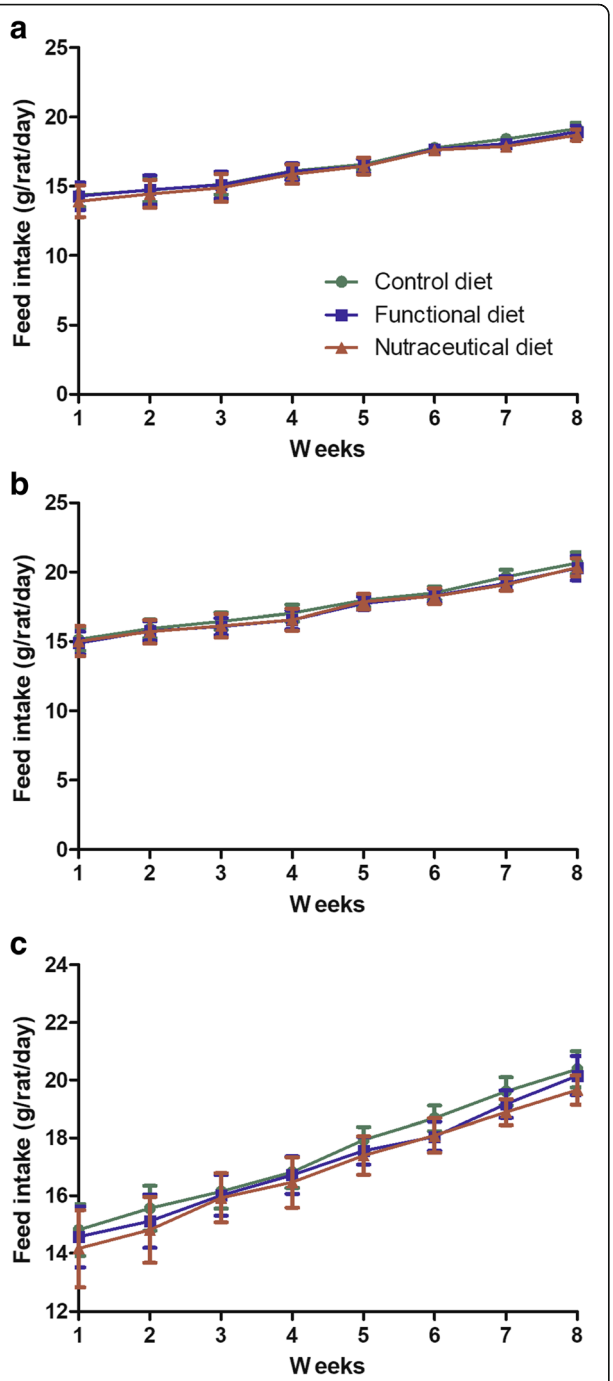

Fig. 1 Feed intake (g/rat/day) of rats increased in all studies i.e. **a**) Normal rats, **b**) hyperglycemic rats, **c**) hypercholestrolemic rats, from 1st to 8th week. Three type of diets were provided in each study; control diet, Functional diet (prepared by conventional solvent extraction method-CSE) and Nutraceutical diet (prepared by supercritical fluid extraction method-SFE)

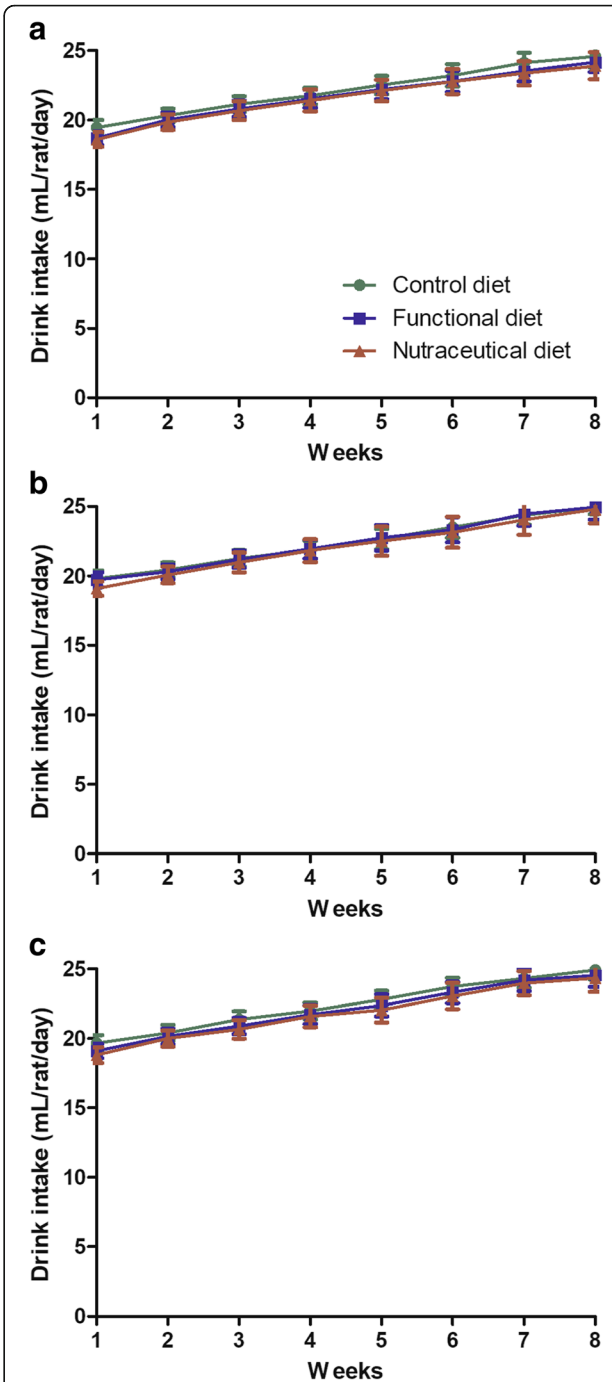

Fig. 2 Drink intake (mL/rat/day) of rats increased in all studies i.e. **a**) Normal rats, **b**) hyperglycemic rats, **c**) hypercholestrolemic rats, from 1st to 8th week. Three type of diets were provided in each study; control diet, Functional diet (prepared by conventional solvent extraction method-CSE) and Nutraceutical diet (prepared by supercritical fluid extraction method-SFE)

Body weight

The mean squares for body weight depicted significant response with respect to diets and time factor. However, the body weight increased throughout the study as growth was occurring constantly but control groups animals weight was more as compared to nutraceutical diet fed counterparts. In study I (Normal rats), the body weight increased from 135.72 ± 6.47, 134.09 ± 6.57 and

132.89 ± 6.34 to 226.64 ± 11.13, 218.56 ± 10.46 and 208.38 ± 10.01 g/rat/day via control, functional and nutraceutical diets, respectively. Similarly, in case of study II (Hyperglycemic rats) and study III (Hypercholesterolemic rats), the body weight in the corresponding groups raised substantially from 137.89 ± 6.35, 135.74 ± 6.53 and 134.05 ± 6.42 to 237.17 ± 11.12, 225.32 ± 11.07 and 217.16 ± 10.53 and from 143.03 ± 7.01, 140.54 ± 6.94 and 138.16 ± 6.67 to 258.88 ± 11.98, 243.13 ± 11.19 and 232.1 ± 11.23 g/rat/day as demonstrated in Fig. 3.

Hypercholesterolemic assessment

Cholesterol

The statistical interpretation depicted that diets significantly affected cholesterol level in all the three studies; study I (normal rats), study II (hyperglycemic rats) and study III (hypercholesterolemic rats) however, time intervals and interaction showed non-significant variance in study I and II except study III. From Day 1 to 60, higher assuaging effect on cholesterol was observed in nutraceutical diet fed groups with reduction of 5.89, 10.32 and 15.22% however, functional diet also proved effective in mitigating cholesterol but at a lesser extent i.e. up to 4.72, 8.52 and 13.15% in all the three studies (study I, II and III), respectively.

Low density lipoprotein-cholesterol (LDL-c)

The statistical analysis demonstrated significant effect of diets on LDL-c in study I, II and III whilst, time affected considerably in study II and III. From initiation to termination of trial, maximum reduction was noted by nutraceutical diet up to 20.5, 12.64 and 6.93% in study III, II and I followed by functional diet by 18.39, 10.81 and 5.85%, respectively.

High density lipoprotein-cholesterol (HDL-c)

The mean squares expressed non-momentous effects of diets and time interval on HDL-c in study I and II, whereas significant difference was noted in study III with respect to diets. The functional and nutraceutical diets maximally up-regulated serum HDL-c level up to 2.94 and 3.36% (study III) followed by 2.61 and 2.85% (study II) and 1.95 and 2.12 (study I), accordingly.

Triacylglycerol

The statistical analysis showed momentous variations on triacylglycerol due to diets in all the three studies I, II and III. However, time intervals and interactions showed non-significant response throughout the trait. From day 1st to 60th, the decrement in triacylglycerol was viewed by 3.62 and 4.79% (Study I), 6.87 and 8.25% (Study II) and 10.13 and 11.76% (Study III) in functional and nutraceutical diets, respectively.

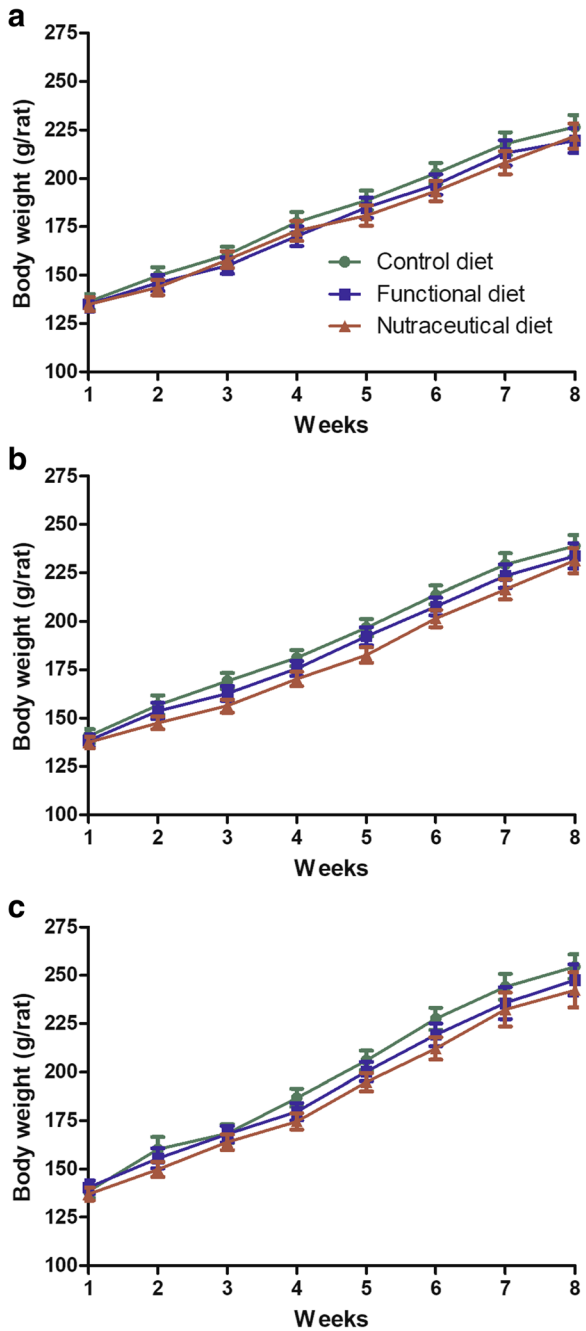

Fig. 3 Body weight (g/rat) of rats increased in all studies i.e. **a**) Normal rats, **b**) hyperglycemic rats, **c**) hypercholestrolemic rats, from 1st to 8th week. Three type of diets were provided in each study; control diet, Functional diet (prepared by conventional solvent extraction method-CSE) and Nutraceutical diet (prepared by supercritical fluid extraction method-SFE)

Hyperglycemic perspectives

Glucose

Mean squares showed significant response of treated diets on glucose levels of rats in study I, II and III, whereas non-significant differences were observed regarding time

intervals and interaction. Throughout the experimentation, the percent reduction in serum glucose in study I, II and III were up to 5.63, 12.71 and 9.29 via nutraceutical diet and 4.27, 11.63 and 7.64 via functional diet, correspondingly.

Insulin

The mean squares portrayed significant impact of diets on insulin level of animals however, non-significant influence was viewed with respect to days and interaction. The percent increase in insulin was observed up to 2.56, 6.75 and 4.32 by functional diet and 3.94, 8.28 and 5.51 through nutraceutical diet in study I, II and III, respectively (Tables 2 and 3).

Hematological aspects

Red blood cells (erythrocytes)

The mean squares explicated non-momentous increment in red blood cells with respect to diets, time intervals and interaction. Means for red blood cells (Tables 4 and 5) via different diets; control, functional and nutraceutical diets were 7.06 ± 0.34, 6.56 ± 0.3 and 6.90 ± 0.32 cells/pL in study I, 5.17 ± 0.24, 4.9 ± 0.22 and 5.22 ± 0.26 cells/pL in study II and 5.82 ± 0.25, 6.10 ± 0.32 and 5.67 ± 0.24 cells/pL in study III. Time factor also affected on red blood cells as 6.82 ± 0.38, 6.84 ± 0.31 and 6.86 ± 0.31 cells/pL at Day 1, 30 and 60 in study I that changed to 5.08 ± 0.24, 5.09 ± 0.24 and 5.12 ± 0.25 cells/pL and 5.84 ± 0.27, 5.86 ± 0.29 and 5.89 ± 0.27 cells/pL in study II and III, accordingly.

White blood cells (lymphocytes)

Statistical scrutiny revealed non-significant response of diets on white blood cells in all the three studies. The control, functional and nutraceutical diets affected on white blood cells (Tables 4 and 5) as 13.22 ± 0.63, 14.14 ± 0.66 and 13.23 ± 0.63 cells/nL in study I, 17.66 ± 0.82, 18.19 ± 0.92 and 17.20 ± 0.78 cells/nL in study II and 14.27 ± 0.65, 15.04 ± 0.73 and 14.29 ± 0.66 cells/nL in study III, respectively. Time factor showed decline in WBC count from 13.63 ± 0.65 (Day 1) to 13.52 ± 0.60 and 13.44 ± 0.63 cells/nL (at Day 30 and 60) in study I, whereas in study II and III the WBC count decreased from 17.82 ± 0.86 to 17.67 ± 0.85 and 17.56 ± 0.83 cells/nL and from 14.66 ± 0.66 to 14.54 ± 0.62 and 14.41 ± 0.59 cells/nL from Day 1 to 30 and 60, respectively.

Platelets (thrombocytes)

Mean squares indicated non-momentous effect of treatments, time and their interaction on platelets. The control, functional and nutraceutical diets enumerated means for platelets (Tables 4 and 5) as 982.16 ± 47.85, 975.87 ± 49.35 and 975.49 ± 47.62 (10^3/μL) in study I, 962.84 ± 45.17, 971.13 ± 46.76 and 974.25 ± 48.13 (10^3/μL) in study II and 940.62 ± 45.27, 961.74 ± 47.31 and 953.79 ± 44.65 (10^3/μL) in study III, respectively. Time intervals showed a non-obvious up-regulation in platelets from Day 1 to 30 and 60; from 971.62 ± 47.61 to 978.47 ± 49.38 and 983.43 ± 48.16 (10^3/μL) in study I, from 962.57 ± 46.53 to 969.61 ± 46.11 and 976.05 ± 47.89 (10^3/μL) in study II and from 943.09 ± 45.79 to 954.92 ± 44.65 and 958.14 ± 46.74 (10^3/μL) in study III, respectively.

Discussion

Previously, Nörnberg et al. [25] observed that EGCG decreases feed intake due to its interaction with leptin receptors. Furthermore, they found green tea catechins effective in controlling obesity by reducing body weight from 103.2 ± 13.85 to 90.36 ± 17.71 g, by up-regulating thermogenesis or modulating lipolysis, by increasing oxygen uptake and by inhibiting catechol *O*-methyl transferase (COMT). It also controls fat synthesis by inhibiting enzymes activity. One of their peers, Ortsäter et al. [26] observed decline in feed intake up to 10–11% in EGCG+diabetic group as compared to control

Table 2 Effect of diets on lipidemic and glycemic parameters of rats in different studies

Studies	Diets	Parameters					
		Cholesterol (mg/dL)	LDL-c (mg/dL)	HDL-c (mg/dL)	Triacylglycerol (mg/dL)	Glucose (mg/dL)	Insulin (μU/mL)
Study I	Control	80.75 ± 3.89^{a}	31.39 ± 1.43^{a}	35.82 ± 1.84	67.08 ± 3.29^{a}	89.97 ± 4.34^{a}	8.32 ± 0.42^{a}
	Functional diet	77.49 ± 3.57^{ab}	30.13 ± 1.46^{ab}	36.88 ± 1.87	65.11 ± 3.17^{ab}	89.19 ± 4.29^{a}	8.56 ± 0.45^{a}
	Nutraceutical diet	76.31 ± 3.39^{b}	28.96 ± 1.32^{b}	37.54 ± 1.56	63.52 ± 3.12^{b}	85.00 ± 4.02^{b}	7.77 ± 0.36^{b}
Study II	Control	99.88 ± 4.68^{a}	47.03 ± 2.24^{a}	39.05 ± 1.97	77.87 ± 3.82^{a}	142.23 ± 6.87^{a}	12.22 ± 0.58^{a}
	Functional diet	93.74 ± 4.32^{b}	44.02 ± 2.12^{b}	38.99 ± 1.87	75.09 ± 3.64^{ab}	127.51 ± 6.18^{b}	11.93 ± 0.59^{ab}
	Nutraceutical diet	91.81 ± 4.11^{b}	42.00 ± 1.95^{b}	40.19 ± 2.01	71.94 ± 3.49^{b}	125.82 ± 6.12^{b}	11.61 ± 0.56^{b}
Study III	Control	150.46 ± 7.18^{a}	62.79 ± 3.04^{a}	53.25 ± 2.28^{b}	100.96 ± 5.06^{a}	101.21 ± 4.86^{a}	10.42 ± 0.52^{ab}
	Functional diet	135.81 ± 6.64^{b}	55.12 ± 2.61^{b}	54.43 ± 2.36^{ab}	92.86 ± 4.56^{b}	96.14 ± 4.59^{ab}	10.75 ± 0.56^{a}
	Nutraceutical diet	131.06 ± 6.35^{c}	53.46 ± 2.54^{c}	56.09 ± 2.65^{a}	90.91 ± 4.49^{b}	93.44 ± 4.41^{b}	9.85 ± 0.48^{b}

Study I (Normal rats); Study II (Hyperglycemic rats); Study III (Hypercholesterolemic rats); Means carrying same letters do not differ significantly

Table 3 Effect of time intervals on lipidemic and glycemic parameters of rats in different studies

Studies	Time intervals (days)	Parameters					
		Cholesterol (mg/dL)	LDL-c (mg/dL)	HDL-c (mg/dL)	Triacylglycerol (mg/dL)	Glucose (mg/dL)	Insulin (μU/mL)
Study I	**1**	79.45 ± 3.78	30.78 ± 1.33	36.47 ± 1.72	66.13 ± 3.29	89.45 ± 4.21	8.13 ± 0.41
	30	78.11 ± 3.45	30.15 ± 1.37	36.76 ± 1.79	65.19 ± 3.15	87.97 ± 4.15	8.22 ± 0.42
	60	76.99 ± 3.40	29.57 ± 1.24	37.01 ± 1.81	64.39 ± 3.12	86.74 ± 4.07	8.31 ± 0.42
Study II	**1**	97.62 ± 4.31	45.86 ± 2.09^{a}	39.11 ± 1.93	76.52 ± 3.74	135.74 ± 6.53	11.69 ± 0.57
	30	95.02 ± 4.15	44.27 ± 2.05^{ab}	39.43 ± 1.95	74.87 ± 3.67	131.67 ± 6.35	11.93 ± 0.60
	60	92.78 ± 4.13	42.93 ± 2.02^{b}	39.70 ± 1.96	73.50 ± 3.57	128.16 ± 6.17	12.14 ± 0.59
Study III	**1**	143.75 ± 7.05^{a}	60.19 ± 2.81^{a}	54.25 ± 2.41	97.40 ± 4.79	99.23 ± 4.69	10.19 ± 0.52
	30	138.83 ± 6.71^{b}	56.95 ± 2.73^{b}	54.60 ± 2.56	94.78 ± 4.63	96.80 ± 4.62	10.35 ± 0.53
	60	134.74 ± 6.34^{c}	54.23 ± 2.62^{c}	54.92 ± 2.67	92.54 ± 4.49	94.76 ± 4.57	10.48 ± 0.54

Study I (Normal rats); Study II (Hyperglycemic rats); Study III (Hypercholesterolemic rats); Means carrying same letters do not differ significantly

diabetic group. Alongside, they assessed that rats fed on EGCG showed less gain in weight during 6 weeks i.e. 65–77% as compared to control rats i.e. 110%. Earlier, Chan et al. [27] found decrease (16%) in water intake by those rats that were relying on 5 g of green tea extract, whereas 15 g of green tea extract resulted in more water intake i.e. 55% during 2-weeks trial. Moreover, they viewed that 5 and 15 g of green tea extract reduced the weight gain in animals by 17 and 18% as compared to control rats due to its association in regulating lipid profile. In another scrutiny, it was observed that water intake increases to a significant level in diabetic rats due to the increased thirst response, resulting higher solute saturation in blood disturbing the electrolyte balance [28]. Further, it is found that green tea catechins are astringent in taste hence compels thirst sensation. This fact is supported in the current study where more water intake was observed by positive control group administered with green tea extract in contrast to normal animals.

Earlier, Yang et al. [29] found reduction in body weight of Sprague Dawley rats by 7.66% by administrating green tea for 25 days. In diabetic control group, increase in body weight is related to increase in triacylglycerol level via sucrose based diet. In contrast, green tea catechins have the ability to suppress glycerol substrate required in the formation of triacylglycerol. In another study, Fukino et al. [30] found ameliorative ability of green tea extract against triacylglycerol formation up to 16.9% by administrating for 2 months.

Later, Elwakkad et al. [17] found reduction in serum cholesterol, LDL-c, HDL-c and triacylglycerol levels in obese rats up to 20–55.1, 37.64–55.41, 53.02–45.8 and 20–55.1% via pure EGCG and 14.95–39.95, 21.77–36.8, 26.13–22.52 and 14.95–39.95% via green tea conventional extract supplementation for 5 to 9 weeks, respectively. The management of hypercholesterolemia is based on increase in cholesterol excretion and decrease in its intestinal absorption. Moreover, green tea extract has the potential to down-regulate cholesterol synthesis by the activation of AMP kinase; master enzyme involved in the homeostasis of cellular energy via phosphorylation or deactivation of HMG-CoA (3-hydroxy-3-methylglutaryl-CoA) reductase, a liver enzyme of mevalonate

Table 4 Effect of diets on hematological parameters of rats in different studies

Studies	Diets	Parameters		
		RBC (cells/pL)	WBC (cells/nL)	Platelets count (10^3/μL)
Study I	Control	7.06 ± 0.34	13.22 ± 0.63	982.16 ± 47.85
	Functional diet	6.56 ± 0.30	14.14 ± 0.66	975.87 ± 49.35
	Nutraceutical diet	6.90 ± 0.32	13.23 ± 0.63	975.49 ± 47.62
Study II	Control	5.17 ± 0.24	17.66 ± 0.82	962.84 ± 45.17
	Functional diet	4.90 ± 0.22	18.19 ± 0.92	971.13 ± 46.76
	Nutraceutical diet	5.22 ± 0.26	17.20 ± 0.78	974.25 ± 48.13
Study III	Control	5.82 ± 0.25	14.27 ± 0.65	940.62 ± 45.27
	Functional diet	6.10 ± 0.32	15.04 ± 0.73	961.74 ± 47.31
	Nutraceutical diet	5.67 ± 0.24	14.29 ± 0.66	953.79 ± 44.65

Study I (Normal rats); Study II (Hyperglycemic rats); Study III (Hypercholesterolemic rats); Means carrying same letters do not differ significantly

Table 5 Effect of time intervals on hematological parameters of rats in different studies

Studies	Time intervals (days)	Parameters		
		RBC (cells/pL)	WBC (cells/nL)	Platelets count (10^3/μL)
Study I	**1**	7.06 ± 0.34	13.22 ± 0.63	982.16 ± 47.85
	30	6.56 ± 0.30	14.14 ± 0.66	975.87 ± 49.35
	60	6.90 ± 0.32	13.23 ± 0.63	975.49 ± 47.62
Study II	**1**	5.17 ± 0.24[b]	17.66 ± 0.82[b]	962.84 ± 45.17
	30	4.90 ± 0.22[a]	18.19 ± 0.92[a]	971.13 ± 46.76
	60	5.22 ± 0.26[c]	17.20 ± 0.78[c]	974.25 ± 48.13
Study III	**1**	5.82 ± 0.25	14.27 ± 0.65	940.62 ± 45.27[a]
	30	6.10 ± 0.32	15.04 ± 0.73	961.74 ± 47.31[c]
	60	5.67 ± 0.24	14.29 ± 0.66	953.79 ± 44.65[b]

Study I (Normal rats); Study II (Hyperglycemic rats); Study III (Hypercholesterolemic rats); Means carrying same letters do not differ significan

pathway and a major biomarker of cholesterol production. Besides, green tea could suppress hepatic FAS (fatty acid synthesis) and intestinal acetyl Co-A cholesterol acetyl transferase hence cause cholesterol esterification, prevent its absorption. Earlier analysis found non-significant increase in HDL-c i.e. 3.14% via 10 cups of green tea per day [31]. Furthermore, it was noticed that green tea consumption at the rate of 450 mL for 4 weeks could lower cholesterol and LDL-c significantly by 5.18 and 6.25% along with non-momentous increment in HDL-c up to 2.1% [32].

Later, Al-Attar and Zari [33] investigated the effect of green tea extract in normoglycemic and hyperglycemic streptozotocin induced albino male rats. After 15 and 30 days, the reduction in cholesterol was observed up to 2.39 and 1.47% in normal rats along with 22.85 and 36.26% decrement in hyperglycemic rats. Moreover, green tea has been found to down-regulate pancreatic lipases that are responsible for triacylglycerol formation resultantly reducing triacylglycerol level up to 3.26% in normal animals while 24.13% in streptozotocin induced diabetic animals. It was also observed in the study that green tea reduces glucose level by 19.89 and 30.88% after 15 and 30 days, respectively in response to insulin stimulated glucose transportation. Afterwards, Haidari et al. [34] found reduction in serum cholesterol, triacylglycerol, LDL-c and glucose levels at two different doses of green tea extract; 100 and 200 mg/kg up to 4.20 and 17.07%, 10.04 and 33.45%, 4.72 and 19.42% and 5.02 and 38.9% along with increment in HDL-c up to 4.59 and 11.94% in diabetic subjects, correspondingly. The underlying mechanisms include reduction in cholesterol absorption by increasing its fecal excretion and reduction in cholesterol biosynthesis by suppressing enzymatic activity. The green tea polycatechins may reduce glucose formation from non-carbohydrate sources (gluconeogenesis) by decreasing phosphoenol pyruvate carboxykinase from entering into gluconeogenesis. Further, it reduces glucose-6-phosphate breakdown to glucose by down-regulating glucose-6-phosphatase.

One of the scientists group, Hasanein et al. [35] tested the effect of 1, 2 and 4 mL green tea extract and found reduction in plasma cholesterol by 7.88, 18.2 and 33.23%, triacylglycerol 13.77, 23.79 and 41.74% and LDL-c up to 11.7, 28 and 46%, respectively by up-regulating their excretion or inhibiting cholesterol formation in the liver cells. The EGCG has a modulatory action on acetyl-CoA carboxylase enzyme i.e. indispensable in the formation of acetoacetyl-CoA in the mevalonate pathway. They also found lipid lowering role of green tea by improving HDL-c level by 27.35% via 1 mL of green tea extract i.e. associated with high paraoxonase 1 (PON1) activity. The reduction of serum glucose concentration up to 20.11, 43.97 and 44.84% via 1, 2 and 4 mL of green tea extract after 4 weeks is associated with up-regulation in insulin level or inhibition of α-amylase enzyme in mouth and intestine i.e. related to glucose reduction and energy release. Besides, they determined decrement in feed intake from 15.25 ± 0.758 g (diabetic group) to 15.485 ± 0.435, 15.226 ± 0.524 and 14.683 ± 0.421 g via 1, 2 and 4 mL of green tea extract, respectively resulted in reduced body weight in comparison to control rat group, especially at higher dose of 4 mL.

LDL-c undergoes oxidative modification by macrophages derived from monocytes forming foam cells. The green tea polyphenols have the ability to inhibit LDL-c oxidation by delaying plaque formation. The resistance to LDL-c oxidation was significantly improved by consuming seven to eight cups of 200 mL green tea containing 300 mg of EGCG and ECG up to 1 week [36, 37]. Furthermore, the effect of green tea extract was evaluated on 12 healthy individuals at the rate of 600 mL of green tea/4 weeks that resulted in reduction of oxidized-LDL-c up to 21.15% [38]. The administration of 4.5 g of green tea was observed to reduce LDL-c up to 10 mg/dL during 4 weeks by inhibiting its absorption in the body [39]. Green tea has catechins particularly EGCG 10 μg/mL has the potential to reduce oxidized LDL up to 65% by capturing reactive oxygen species (ROS), controlling the formation of MDA-LDL (malondialdehyde modified-LDL-c) [40]. Afterwards, Nagao et al. [41] conducted a clinical trial based on two Japanese groups, fed with 22 and 690 mg of catechins per day for 12 weeks. The study resulted in reduction of malondialdehyde-modified LDL (13.96%) due to thermogenic effect of EGCG and catechins. Moreover, green tea catechins at the rate of 690 mg/day resulted in 7.5% increase in insulin release from 67.5 ± 5.2 to 72.6 ± 10.5 picomol/L. However, non-significant effects were recorded on blood indices as indicated in the current research.

Mehra et al. [19] found decrease in cholesterol synthesizing enzymes up to 11, 25 and 20% in high cholesterol plus high sugar diet fed animals by supplementing catechins based diet for 4, 8 and 12 weeks, respectively. Alongside, they also found decrease in LDL-c along with increase in HDL-c. This is because green tea overcomes the detrimental symptoms associated with high fat diet, inducing lipogenesis by stimulating acetyl-CoA carboxylase and fatty acid synthase (FAS). Another mechanism involved is the induction of thermogenesis process by green tea i.e. consuming oxygen and increasing energy expenditure by 3 to 4%. In the similar study, they expounded therapeutic effects of green tea against type 2 diabetes by enhancing insulin activated GLUT4 transport. The obese rats were having 57% high plasma glucose concentration as compared to normal rats. However, catechin+high fat and sugar diet showed 9.6% reduction as compared to positive control. The mechanism explains that EGCG has insulin potentiating activity that increases glucose uptake by fat cells however, decreases glucose absorption through intestinal cells by preventing Na-dependent glucose transportation (SGLT1).

Another analysis investigated the therapeutic potential of different doses of EGCG (0.25, 0.5 and 1%) that responded dose dependent decline in glucose up to 2.42, 21.6 and 36.87% in mice and 15.8% in rats at 0.5% dose. They also noted increment in insulin response in diabetic and obese subjects from 0.14 (control) to 0.96 nmol/L by a dose of 1% EGCG. Likewise, they found triacylglycerol suppression by 26, 29 and 63% via 0.25, 0.5 and 1% EGCG however, 18.98% decrease in rats at the rate of 0.5% EGCG. The reduction in glycerol-3-phosphate acyl transferase could be a possible inhibitory mechanism involved [42]. Furthermore, Kao et al. [43] indicated reduction in serum cholesterol from 83 ± 0.8 to 69 ± 2.2 mg/dL in Sprague Dawley rats within 7 days. However, triacylglycerol and glucose level was observed to reduce by 45.8 and 31.81%. EGCG is associated with reduced triacylglycerol level by reducing the fatty acid synthesizing enzymes or by increasing the fecal excretion of sterols. Additionally, they studied the effect of two dominant catechins of green tea on red blood cells count (10^6/μL) and found increment from 6.51 ± 0.11 (control) to 7.40 ± 0.23 via ECG and 7.90 ± 0.15 via EGCG. The higher RBC content has been positively associated with increased hemoglobin level from 13.7 ± 0.46 g/dL (control) to 15.5 ± 0.45 g/dL via ECG and16.8 ± 0.46 g/dL with EGCG. They also observed decrease in WBC (10^3/μL) from 11.03 ± 1.39 to 9.40 ± 1.00 by ECG and 9.92 ± 0.19 via EGCG. It was also found in the same study that neutrophils (anti-inflammatory) increased but other indices of WBC including lymphocytes, monocytes and esinophils decreased non-significantly. Moreover, ECG and EGCG resulted in increase of platelets from 950 ± 80 to 1325 ± 96 (10^3/μL) via ECG and 1942 ± 59 (10^3/μL) through EGCG. Later, Batista et al. [44] studied the decrease in total cholesterol by 3.9% and LDL up to 4.5% in 33 dyslipidemias during 8 weeks by feeding green tea dry extract in-combination with low cholesterol diet. However, reduction in triacylglycerol level was viewed after 16 weeks.

In an animal trial, Islam and Choi [45] observed the significant reduction in lipid and glucose profile of diabetic Sprague Dawley rats at higher dose of green tea diet (2%) in contrast to low green tea diet (0.5%). The green tea polyphenols hence reduced the LDL-c and triacylglycerol up to 24 and 46.66% in diabetic rats after 4 weeks, respectively. The triacylglycerol reduction is attributed to decreased triacylglycerol absorption in the gut system. Furthermore, green tea possesses insulinotropic (insulin like) effects, raising insulin secretion. They found lower dose of green tea more effective in managing the body weight. In the study, it was also clarified that higher EGCG consumption resulted in reduced feed consumption as compared to positive control diabetic animals. On the other hand, they noticed higher feed intake in diabetic rats relying on lower dose (0.5%) of green tea that resulted in higher liver glycogen and liver weight. Besides, higher amounts of EGCG (i.e. 2%) may cause cytotoxicity hence decreasing the desire for food. Later, Sabahelkhier et al. [46] found that aqueous extract of green tea (12%) significantly reduced body weight 47.6%, cholesterol 59.9%, triacylglycerol 32% and glucose 47.61% in American white rats. The reduction in lipidemic parameters cut-down the risk of cardiovascular events and increase in glucose uptake by fat cells due to insulin potentiating effect is attributed to green tea extract. Further investigation ascribed the positive role of green tea against renal dysfunctions in response to hyperlipidemic and hyperglycemic conditions.

Earlier findings supported that green tea extract (500 mg/day) improved insulin by 4.89% hence correcting type-2 diabetes [47]. The anti-insulin resistant activity of green tea extract especially EGCG has been studied in rats. It has been found that green tea extract at the rate of 1 to 2 g/kg diet for 6 weeks possesses insulin potentiating activity up to 5.6% in experimental rats or it might be associated with correction of impaired β-cell functioning. Thus, green tea extract balances between hyperinsulinemia and hyperglycemia for the homeostatic control of blood glucose level [48]. Further analysis showed that EGCG 200 mg/kg/day for 12 weeks reduced plasma glucose load up to 15% by increasing insulin sensitivity up to 13% [49]. Similarly, Chan et al. [50] elucidated 15% increase in insulin level by supplementing green tea to 34 obese Chinese female subjects for 3 months.

Additionally, Chengelis et al. [51] observed the safety of green tea catechins on the experimental animals. The RBCs (10^6/μL) raised from 7.89 ± 0.264 (control) to 8.30 ± 0.432, 8.56 ± 0.436, 8.63 ± 0.337 and 8.15 ± 0.388 at the rate of 500, 1000, 2000 mg/kg/day of green tea and 2000 mg/kg/day of decaffeinated green tea, respectively as observed in the current investigation. The decaffeinated green tea extract at the rate of 2000 mg/kg/day during 28 days resulted in reduced WBCs from 10.27 ± 2.828 (control) to 10.17 ± 1.518 (× 10^3/μL) i.e. decrease up to 0.97% in male and 2.7% in female subjects from 8.77 ± 1.420 to 8.53 ± 1.518 (× 10^3/μL). This has resulted in non-significant reduction due to the non-momentous rise in neutrophils and monocytes only. Although, most WBC indices like lymphocytes, esinophils and basophils showed a decreasing trend. Moreover, the platelet count (10^3/μL) observed an inclining trend from 1013 ± 112.8 (control) to 1013 ± 174.5 in male and from 1125 ± 137.7 (control) to 1159 ± 254 in female subjects as viewed in the present study. However, green tea extract at 500 and 1000 mg/kg/day resulted in increased platelet count up to 1154 ± 139.4 and 1046 ± 158.6 and 1139 ± 97.0 and 1162 ± 150.8 (10^3/μL) in males and female subjects as compared to control, respectively. One of their peers, Matsuyama et al. [52] carried out an experimental trial on 40 obese Japanese children administrated with green tea at the rate of 75 (control group) and 576 (catechin group) mg/day for 24 weeks that showed increased RBCs in both groups from 492.7 ± 7.4 (× 10^{10}/L) at 0 day to 496.7 ± 8.7 (× 10^{10}/L) at 24th week in catechin group and from 490.2 ± 7.6 (× 10^{10}/L) at 0 day to 496.9 ± 7.3 (× 10^{10}/L) at 24th week in control group by increasing the hemoglobin content. They also found increase in platelets content at the rate of 75 mg/day during 24 weeks from 32.5 ± 1.48 to 33.05 ± 1.57 × 10^{10}/L. Another meta-analysis on rats and dogs confirmed the non-toxic effects of green tea at the rate of 500 mg/kg/day after 13 weeks. The RBCs were found to increase from 7.62 ± 0.468 10^6/μL (control) to 7.63 ± 0.467 10^6/μL at the rate of 500 mg/kg/day in rats with the non-significant increase in hemoglobin content. Moreover, EGCG at the rate of 500 mg/kg/day after 13 weeks resulted in reduction of WBCs 3.29% from 9.1 ± 1.55 to 8.4 ± 1.331 (× 10^3/μL) in female rats. However, increase in platelets from 1060 ± 78 (control) to 1127 ± 113 (× 10^3/μL) was viewed in experimented rats at the rate of 150 mg EGCG/kg/day [53].

Conclusion

In a nutshell, green tea extract has shown the ability to reduce hyperglycemia and lipidemic profile except HDL-c, alongside green tea catechins portrayed insulin mimetic effects. Comparison of extracts clarified that extract achieved via supercritical fluid extraction mode (nutraceutical diet) was more effective against altered biomarkers as compared to conventional solvent extracted counterpart (functional diet). Conclusively, the study proved that extracted green tea polycatechins could be introduced in routine menu to attain the optimal health benefits and to fight against lifestyle mediated maladies.

Abbreviations

CSE: Conventional Solvent Extraction; FAS: Fatty Acid Synthesis; HDL-c: High Density Lipoproteins-Cholesterol; HMG-CoA: 3 Hydroxy-3-Methylglutaryl Coenzyme-A Reductase; IDDM: Insulin Dependent Diabetes Mellitus; LDL-c: Low Density Lipoprotein-Cholesterol; MDA-LDL: Malondialdehyde Modified-LDL-c; NIDDM: Non-Insulin Dependent Diabetes Mellitus; RBC: Red Blood Cell; SFE: Supercritical Fluid Extraction; SGLT1: Na-Dependent Glucose Transportation; WBC: White Blood Cell

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Availability of data and materials

Not applicable.

Authors' contributions

FA designed the project under the supervision of MSB, afterwards KAA helped in extraction procedures and AB in conducting animal trials. FA, HARS prepared the final manuscript. All authors read and approved the final manuscript.

Ethics approval

Ethics approval was provided by the head of the NIFSAT-UAF, Pakistan, by reviewing the plans of Animal Experimentation Ethics Committee, UAF. The care of animals during experimentation were as per the instructions provided by the committee and the university.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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